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Am J Physiol Endocrinol Metab 299:E215-E224, 2010. First published 18 May 2010;
doi:10.1152/ajpendo.00098.2010

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Submitted 11 February 2010; accepted in final form 9 May 2010

Brandt N, De Bock K, Richter EA, Hespel P. Cafeteria diet-induced insulin resistance is not associated with decreased insulin signaling or AMPK activity and is alleviated by physical training in rats. *Am J Physiol Endocrinol Metab* 299: E215–E224, 2010. First published May 18, 2010; doi:10.1152/ajpendo.00098.2010.—Excess energy intake via a palatable low-fat diet (cafeteria diet) is known to induce obesity and glucose intolerance in rats. However, the molecular mechanisms behind this adaptation are not known, and it is also not known whether exercise training can reverse it. Male Wistar rats were assigned to 12-wk intervention groups: chow-fed controls (CON), cafeteria diet (CAF), and cafeteria diet plus swimming exercise during the last 4 wk (CAF_{TR}). CAF feeding led to increased body weight (16%, $P < 0.01$) and increased plasma glucose ($P < 0.05$) and insulin levels ($P < 0.01$) during an IVGTT, which was counteracted by training. In the perfused hindlimb, insulin-stimulated glucose transport in red gastrocnemius muscle was completely abolished in CAF and rescued by exercise training. Apart from a tendency toward an ~20% reduction in both basal and insulin-stimulated Akt Ser^{473} phosphorylation ($P = 0.051$) in the CAF group, there were no differences in insulin signaling (IR $Tyr^{1150/1151}$, PI 3-kinase activity, Akt Thr^{308}, TBC1D4 Thr^{642}, GSK3-α/β $Ser^{21/9}$) or changes in AMPKα1 or -α2, GLUT4, Munc18c, or syntaxin 4 protein expression or in phosphorylation of AMPK Thr^{172} among the groups. In conclusion, surplus energy intake of a palatable but low-fat cafeteria diet resulted in obesity and insulin resistance that was rescued by exercise training. Interestingly, insulin resistance was not accompanied by major defects in the insulin-signaling cascade or in altered AMPK expression or phosphorylation. Thus, compared with previous studies of high-fat feeding, where insulin signaling is significantly impaired, the mechanism by which CAF diet induces insulin resistance seems different.

adenosine 5′-monophosphate-activated protein kinase; diet; exercise; glucose transport

OBESITY AND INSULIN RESISTANCE are hallmarks of type 2 diabetes (11). A widely used animal model to study insulin resistance is high-fat feeding. Rodents fed a high-fat diet (HFD) rapidly develop decreased insulin action and impaired activation of the insulin-signaling pathway (15, 17, 18, 36, 42). However, high-fat feeding may be considered a radical dietary intervention, whereas a cafeteria diet (26) consisting mainly of a palatable diet with a more balanced caloric composition (15% protein, 69% carbohydrates, 16% fat) better resembles a Western diet (14a, 20). Feeding a palatable cafeteria diet in rats results in obesity and glucose intolerance (25, 26, 37), but the underlying mechanisms are not known. Furthermore, it is not known whether exercise training can alleviate cafeteria diet-induced insulin resistance. In HFD-fed rats, serine phosphorylation of IRS-1 was increased three- to fourfold, which was correlated with impaired IRS-1 tyrosine phosphorylation and Akt serine (Ser^{473}) phosphorylation (36, 42). This inhibition of insulin signaling was associated with activation of the stress-activated NF-κB inflammatory pathway shown by increases in NF-κB-inducing kinase protein, NF-κB p65 total protein, and IKKβ activity. In addition, phosphorylation of JNK, a molecule known to serine phosphorylate IRS-1, was increased by HFD (36). Thus HFD is believed to induce low-grade inflammation and thereby interfere with proximal insulin signaling via inhibition of IRS-1 function.

AMP-activated protein kinase (AMPK) is a fuel-sensing enzyme that responds to reductions in cellular energy charge (increased AMP/ATP ratio) (8, 43) such as during exercise, hypoxia, or prolonged starvation (14, 21). In some animal models of insulin resistance activity of AMPK is reduced (7, 44), and it has been hypothesized that dysregulation of AMPK activity (decreased expression or activation) might contribute to development of insulin resistance and other metabolic abnormalities (28). In humans, some studies have shown decreased AMPK activity in muscle of individuals with obesity and insulin resistance (3, 4, 31), whereas others have shown no abnormalities (16, 32). Exercise training is a significant physiological stimulus known to activate AMPK in skeletal muscle and has been shown to effectively counteract diet/lipid-induced insulin resistance (9, 29, 39, 41). Moreover, treatment with antidiabetic agents such as metformin and thiazolidinediones activates AMPK in skeletal muscle (3, 22).

The purpose of this study was to investigate whether a period of cafeteria dieting induces obesity and insulin resistance on whole body level as well as in skeletal muscle and to elucidate the underlying molecular mechanisms. Moreover, the potential role of exercise in counteracting this development was investigated. The results presented in this study are part of a larger study on the relation between excess energy intake, exercise training, and insulin signaling in skeletal muscle, and some of the basic characteristics of the rats have been published before (37).

METHODS

Animals

Male Wistar rats weighing 220–250 g were obtained from Katholieke Universiteit Leuven University Breeding Centre. All animals were given ad libitum access to food and water and were caged

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individually and kept on a 12:12-h light-dark cycle at 22°C. In all experimental groups, daily food intake and body weight were registered. This study protocol was approved by the Animal Ethics Committee of the University of Leuven (Belgium).

Study Protocol

Rats were divided randomly into one of three 12-wk intervention groups distinguished by dietary composition and application of exercise training. *Group 1* [chow-fed controls (CON); $n = 17$] was used as a control group fed a normal pellet diet containing 11% fat, 26% protein, and 63% carbohydrate. *Groups 2*($n = 13$) and *3* ($n = 14$) were fed a "cafeteria-style" diet (CAF) (26) containing 330 g/kg ground Muracon-G pellets (Carfil Quality, Oud-Turnhout, Belgium), 330 g/kg Nestlé full-fat sweetened condensed milk, 70 g/kg sucrose, and 270 g/kg water. This cafeteria diet provided 15% of its energy content as protein, 69% as carbohydrate and 16% as fat (37). This diet was designed to be highly palatable by preparing it as a paste that the rats seemed to like, and thus they ate relatively large amounts of it. Furthermore, *group 3* was subjected to exercise training during the last 4 wk of the 12-wk diet period (CAF_{TR}). The rats performed 1 h of swimming exercise 5 times/wk in water maintained at 32–35°C. To ensure continuous swimming, each rat had a load attached to the tail equivalent to 2% of its body weight.

Intravenous Glucose Tolerance Test

Following the above-described 12-wk period of interventions, rats were anesthesized by an intraperitonal injection (0.2 ml/100 g body wt) of a mixture containing 50% Ketalar (50 mg/ml ketamine; Pfizer, Brussels, Belgium), 25% Rompun (2% xylazine; Bayer, Leverkussen, Germany), and 25% atropine (0.5 mg/ml) and prepared surgically for an intravenous glucose tolerance test (IVGTT) by insertion of a catheter into the left vena jugularis. Rats were then allowed to recover from the anesthesia and were subjected to the IVGTT the next day, which for the exercise-trained group was 24 h after the last swimming bout. After an overnight fast (16–18 h), a jugular catheter with 1 g glucose/kg body wt was injected into conscious rats, using a 30% (wt/vol) glucose solution in 0.9% (wt/vol) saline. For blood glucose determination, tail blood samples (25 μl) were collected in Na-heparinzed capillaries before the glucose injection and 5, 10, 15, 30, 60, 90, and 120 min afterward. Blood glucose concentration was measured immediately after, using an Analox GM7 (Analox Instruments, London, UK) glucose analyzer. For plasma insulin determination, tail blood samples (150 μl) were collected in Na-heparinzed capillaries before the glucose injection and 10, 30, and 90 min afterward. Blood was immediately centrifuged (13,000 *g*, 3 min) at 4°C using a Beckman Coulter Allegra 64 centrifuge (Fullerton, CA), and the supernatant was stored at −80°C for later analysis. After completion of the IVGTT, only the external part of the catheter was removed to prevent the rats from pulling it out.

Hindlimb Perfusion Procedure

Rats were allowed to recover from the IVGTT and received their usual diet for 4 nights, during which daily food intake was registered. The exercise-trained group resumed swimming (2 bouts) after the IVGTT and had its last bout 48 h before perfusion. Following an overnight fast, rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body wt). Hindlimb perfusion was performed as described by Wojtaszewski et al. (38).

All perfusions were carried out using a cell-free perfusate consisting of Krebs-Ringer bicarbonate buffer solution, 4% bovine serum albumin (fraction V; Sigma), 0.15 mM pyruvate, and 4.2 IU/ml heparin and no (basal glucose transport) or 200 μU/ml insulin (insulin-stimulated glucose transport). During perfusion the arterial perfusate was continuously gassed with a mixture of 95% O_2 and 5% CO_2, yielding average O_2 pressure and pH of 630 ± 13 mmHg and 7.33 ± 0.02, respectively. The temperature of the arterial perfusate was ~35°C. Following surgical preparation, the hindlimbs were allowed to recover for 15 min with perfusate recirculation at a flow of 20 ml/min. The initial 150 ml of glucose-free perfusate passing through the hindlimb was discarded. Thereafter, the glucose-free recovery perfusate was exchanged for perfusate containing 8 mM 2-deoxy-D-glucose and 1 mM mannitol, including 2-[2,6-^{3}H]deoxy-D-glucose (specific activity 51.0 $mCi \cdot mmol^{-1} \cdot l^{-1}$; Amersham Radiochemicals, GE Healthcare, Uppsala, Sweden) and D-1-[^{14}C]mannitol (specific activity 87.0 $mCi \cdot mmol^{-1} \cdot l^{-1}$; Amersham Radiochemicals, GE Healthcare) yielding activities of 0.075 μCi/ml for ^{3}H and 0.050 μCi/ml for ^{14}C. The isotopes reached the hindlimbs exactly at the end of the 15-min recovery period. To secure a constant specific activity for 2-deoxy-D-glucose in the arterial perfusate, one-way perfusion at a flow of 20 ml/min was started. Following 10 min of isotopic perfusion, circulation was stopped. Immediately after this, the medial superficial part and the deep part of the medial head of m. gastrocnemius were dissected from both hindlimbs, freed from any visible connective tissue and blood, and snap-frozen with aluminum clamps cooled in liquid nitrogen and stored at −80°C. These two muscle parts were selected because they represent primarily white glycolytic and red oxidative and glycolytic fibers, respectively (2).

Daily Food Intake

Every morning rats were provided with either freshly prepared cafeteria diet or normal pellets ad libitum. To calculate the daily food intake, residual food was weighed on the next day. Furthermore, body weight was measured on a weekly basis.

Blood Biochemistry

Whole blood glucose concentration was determined in duplicate on an Analox GM7 glucose analyzer. Plasma insulin was assayed by a sensitive rat insulin RIA kit (Linco Research).

Glucose Transport

Measurement of muscle glucose transport was performed as described (38). In short, muscle uptake of 2-[2,6-^{3}H]deoxy-D-glucose was measured in perchloric acid extracts and corrected for label in the extracellular space as determined by the ^{14}C counts for mannitol. From the intracellular accumulation of 2-[2,6-^{3}H]deoxy-D-glucose, the rate of glucose transport was calculated using a specific activity of hexose

Table 1. *Body mass and epididymal fat pad mass in control rats and rats receiving cafeteria diet in the absence or presence of exercise training*

	CON		CAF		CAF_{TR}	
	0 wk	12 wk	0 wk	12 wk	0 wk	12 wk
Body mass, g	218 ± 3 ($n = 9$)	474 ± 16* ($n = 9$)	217 ± 1 ($n = 13$)	522 ± 9*‡§ ($n = 13$)	218 ± 2 ($n = 14$)	470 ± 7* ($n = 14$)
Epididymal fat pad mass, g		7.3 ± 0.8 ($n = 3$)		15.2 ± 1‡§ ($n = 10$)		10.6 ± 0.9† ($n = 10$)

Values are means ± SE. CON, control rats; CAF, cafeteria diet-fed rats in the absence of training; CAF_{TR}, cafeteria diet-fed rats in the presence of training. *$P < 0.01$ between 0 and 12 wk; †$P < 0.05$, different compared with CON; ‡$P < 0.01$, different compared with CON; §$P < 0.01$ different compared with CAF_{TR}. Body mass data have been published elsewhere (37).

Table 2. *Daily food intake, fasting plasma glucose, and fasting insulin values in control rats and rats receiving cafeteria diet in the absence or presence of exercise training*

	CON	CAF	CAF_{TR}
Daily energy intake, kJ	349 ± 5 (n = 10)	392 ± 7 (n = 13)†‡	363 ± 5 (n = 14)
Carbohydrates, E%	63.2	69.5	69.5
Sugar, g/day	1.72 ± 0.02	11.2 ± 0.2*‡	10.4 ± 0.13*
Starch, g/day	11.3 ± 0.16	4.92 ± 0.09*‡	4.56 ± 0.06*
Proteins			
E%	25.5	14.7	14.7
g/day	5.25 ± 0.08	3.41 ± 0.06*‡	3.16 ± 0.04*
Fats			
E%	11.3	15.8	15.8
Unsaturated, g/day	0.82 ± 0.01	0.8 ± 0.01‡	0.74 ± 0.01*
Saturated, g/day	0.28 ± 0.01	0.88 ± 0.02*‡	0.82 ± 0.01
Fasting glucose, mmol/l	3.3 ± 0.15 (n = 12)	3.66 ± 0.16 (n = 21)	3.59 ± 0.17 (n = 15)
Fasting insulin, ng/ml	0.9 ± 0.14 (n = 12)	2.52 ± 0.25 (n = 22)†§	1.5 ± 0.12 (n = 16)

Values are means ± SE. *$P < 0.05$, different compared with CON; †$P < 0.01$, different compared with CON; ‡$P < 0.05$, different compared with CAF_{TR}; §$P < 0.01$, different compared with CAF_{TR}. Daily energy intake data have been published elsewhere (37).

determined by the hexose concentration and 2-[2,6-^{3}H]deoxy-D-glucose counts in the perfusate. Radioactivity was measured in a liquid scintillation counter (1600 TR Liquid Scintillator Analyzer; Packard).

Skeletal Muscle Analysis

Muscle glycogen. For determination of glycogen content, muscle samples from the right hindlimb were freeze-dried for 36 h at −50°C. Muscle glycogen content of the freeze-dried samples (2–3 mg) was determined fluorometrically by the hexokinase method after acid hydrolysis (19a).

Homogenization. In brief, 20 mg of freeze-dried muscle was homogenized in ice-cold buffer containing 10% glycerol, 20 mM Na-pyrophosphate, 150 mM NaCl, 50 mM HEPES (pH 7.5), 1% NP-40, 20 mM β-glycerophosphate, 10 mM NaF, 2 mM EDTA (pH 8.0), 2 mM PMSF, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 2 mM Na_3VO_4, and 3 mM benzamidine. Homogenates were rotated end over end for 1 h at 4°C before being centrifuged for 30 min (17,500 *g*, 4°C). Protein content in the supernatant was measured by the bicinchoninic acid method (Pierce).

Immunoblotting. Total protein and phosphorylation of relevant proteins were determined in muscle lysates by SDS-PAGE, followed by immunoblotting. Phosphorylation of Akt Ser^{473}, IR Tyr$^{1150/1151}$, JNK Thr^{183}/Tyr^{185}, and AMPK Thr^{172} as well as GSK-3 α/β Ser$^{21/9}$ was measured using phosphospecific antibodies from Cell Signaling Technology (Beverly, MA). Total protein levels of Akt1 and Akt2 were measured using antibodies from Cell Signaling Technology. Phosphorylation of Akt Thr^{308} was measured using a phosphospecific antibody from Upstate Biotechnology (Lake Placid, NY). Total protein levels of hexokinase II (HKII) and GLUT4 were measured using antibodies from Alpha Diagnostic International (San Antonio, TX) and Affinity Bioreagents (Golden, CO), respectively. Total protein levels of Munc18c and syntaxin 4 were measured using antibodies from Santa Cruz Biotechnology (Santa Cruz, CA). Total protein levels of AMPKα1 and -α2 were measured using antibodies kindly donated by D. G. Hardie (University of Dundee, Dundee, UK). Phosphorylation of TBC1D4 Thr^{642} was measured using a phosphospecific antibody kindly donated by D. E. James (Garvan Institute of Medical Research, Melbourne, Australia).

Immunoprecipitation of Akt

Akt1 was immunoprecipitated from 100 μg of lysate from CON, CAF, and CAF_{TR} samples with an Akt1 antibody from Cell Signaling Technology. After an overnight incubation at 4°C, the immunoprecipitate was washed twice in Tris-buffered saline (pH 7.5) with 10% glycerol, 20 mM Na-pyrophosphate, 150 mM NaCl, 50 mM HEPES (pH 7.5), 1% NP-40, 20 mM β-glycerophosphate, 10 mM NaF, 2 mM EDTA (pH 8.0), 2 mM PMSF, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 2 mM Na_3VO_4, and 3 mM benzamidine.

Insulin receptor substrate-1-associated phosphatidylinositol-3 kinase activity

Insulin receptor substrate-1 (IRS-1) was immunoprecipitated from 300 μg of lysate with a specific antibody kindly provided by Dr. Ken Siddle (University of Cambridge, Cambridge, UK). After an overnight

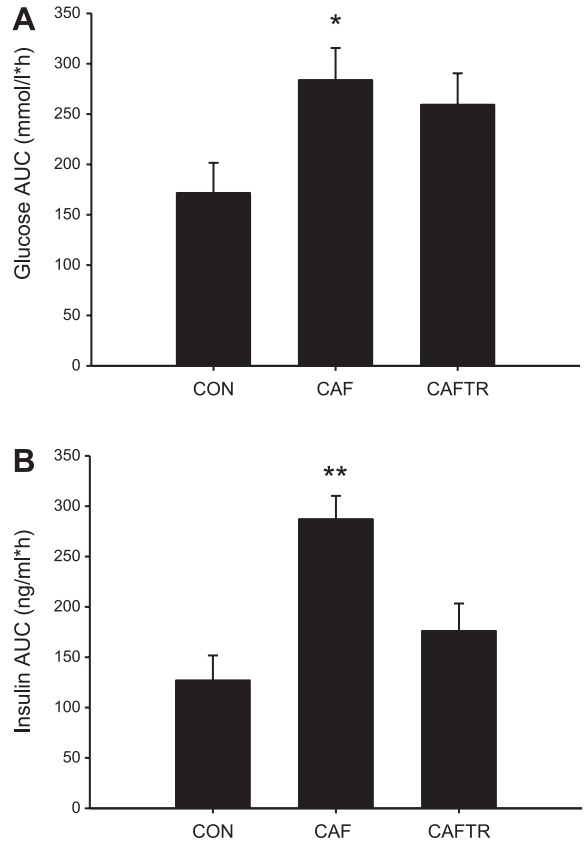

Fig. 1. Plasma glucose and plasma insulin during an intravenous glucose tolerance test (IVGTT) area under the curve (AUC). IVGTT was performed in control rats (CON) and cafeteria diet-fed rats in the absence (CAF) and in the presence of training (CAF_{TR}). Plasma glucose AUC (*A*) and plasma insulin AUC (*B*) during a 120-min IVGTT. Values are means ± SE; n = 7–13. *$P < 0.05$, different compared with CON; **$P < 0.01$ different compared with CON.

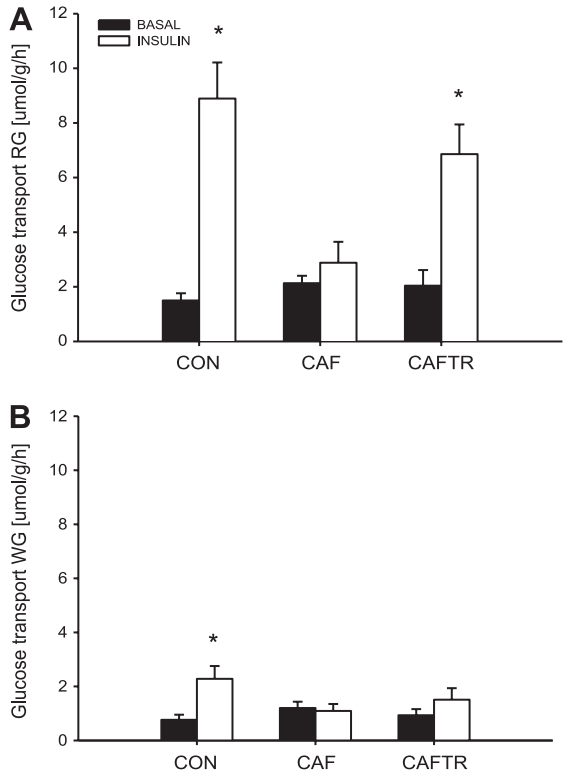

Fig. 2. Basal (black bars) and insulin-stimulated (open bars) glucose transport in red (RG; *A*) and white gastrocnemius (WG; *B*) muscle during hindlimb perfusion. Muscles were obtained from CON, CAF, and CAF_{TR} rats. Values are expressed as means ± SE; $n = 6–14$. *$P < 0.05$, different compared with basal. Glucose transport data have been published elsewhere (37).

incubation at 4°C, the immunoprecipitate was washed twice in PBS (pH 7.5) with 1% NP-40 and 100 μM Na_3VO_4 and twice in Tris-buffered saline (pH 7.5) with 100 mM NaCl, 1 mM EDTA, and 100 μM Na_3VO_4 and left with 50 μl of the last wash buffer. Ten microliters of 100 mM $MgCl_2$ and 10 μg of L-α-phosphatidylinositol (PI; Sigma-Aldrich) were added, and the samples were left at room temperature for 15 min. The reaction was started by the addition of 10 μl of reaction mixture [8.25 mM Tris (pH 7.5), 825 μM EDTA, 6 mM $MgCl_2$, 440 μM ATP, and 100 pM ^{33}P-ATP (0.6 μCi); PerkinElmer]. The reaction ran for 15 min at 30°C and was stopped by the addition of 10 μl of 5 N HCl. Then 180 μl of methanol-chloroform (1:1) was added, and samples were shaken vigorously for 1 min and centrifuged for 90 s. Eighty microliters of the lower organic fraction was transferred to a new tube, and 50 μl were spotted onto a TLC Silica gel (Merck). The chromatographic separation ran for 45 min, after which the TLC gel was dried and exposed in a PhosphorImager Cassette for 48 h before scanning in a Molecular Dynamics STORM scanner (Struers Kebo Laboratory).

Statistical Analysis

Data are expressed as means ± SE. Statistical evaluations were performed by two-way ANOVA with repeated measurements using the Bonferroni test for post hoc comparisons when appropriate. Differences between groups were considered statistically significant if $P < 0.05$.

RESULTS

Some of the basic descriptive data from these rats have been published before (37). For clarity, a few data are repeated here, and in the tables and figures it is clearly stated whether data have been published before.

Body Mass, Epididymal Fat Pad Mass, Daily Energy Intake, and Substrate Measurements

There were no detectable differences in animal body weights prior to the interventions. During the 12 wk animals in all groups gained weight, and after 12 wk the CAF group was

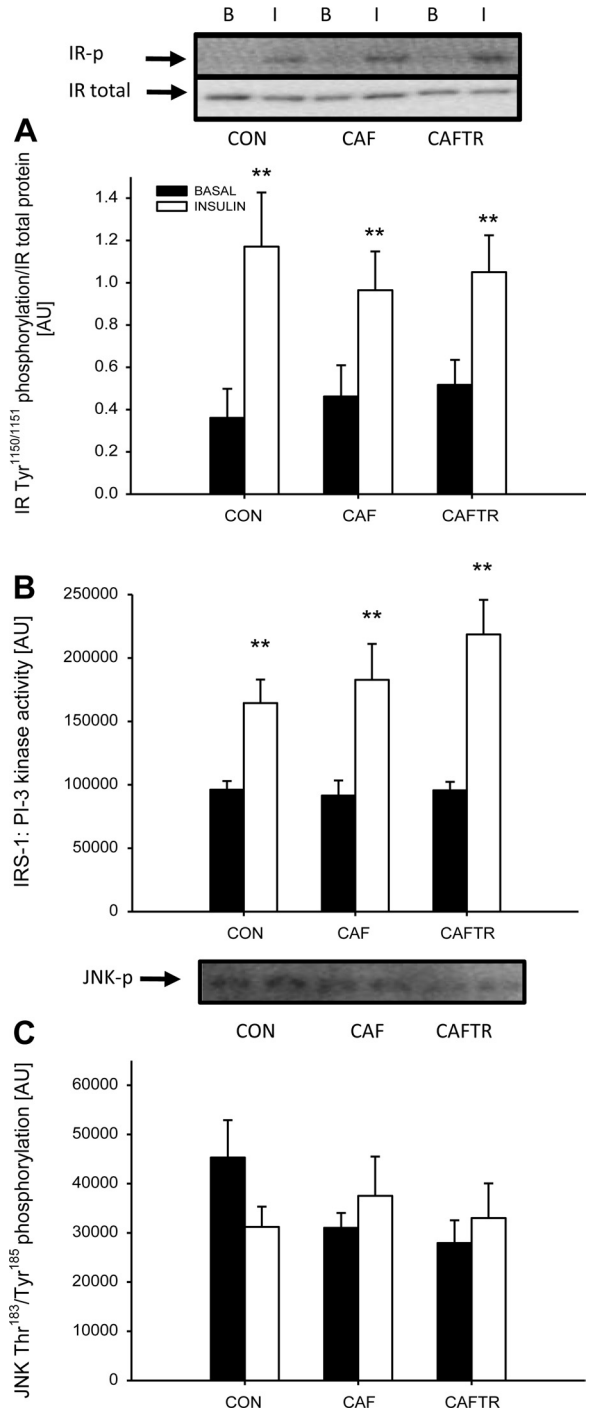

Fig. 3. Insulin receptor (IR) Tyr^{1151} phosphorylation relative to total IR protein content (*A*), IR substrate (IRS)-1-associated phosphatidylinositol (PI) 3-kinase activity (*B*), and JNK Thr^{183}/Tyr^{185} phosphorylation (*C*) in basal (black bars; B) and insulin-stimulated (open bars; I) RG muscle from a perfused hindlimb. Muscles were obtained from CON, CAF, and CAF_{TR} rats. Values are means ± SE; $n = 5–9$. **$P < 0.01$ compared with basal. AU, arbitrary units.

significantly heavier (~10%, $P < 0.01$) than the two other groups (Table 1). Twelve weeks of cafeteria diet feeding led to development of significantly larger epididymal fat pads in both the CAF and the CAF_{TR} groups (105 and 43%, respectively, $P < 0.01$, $P < 0.05$) compared with CON, although CAF_{TR} has smaller pads than CAF ($P < 0.01$) (Table 1). The average daily energy intake during the 12 wk was significantly larger (~12%, $P < 0.05$) in the CAF group compared with the two other groups (Table 2). After 12 wk of intervention there was no difference in fasting glucose between groups, but fasting insulin was significantly higher in the CAF group compared with CON and CAF_{TR} (180 and 68%, respectively, $P < 0.01$; Table 2).

In Vivo Glucose Tolerance: IVGTT

During an IVGTT, fasting plasma glucose was similar among groups, whereas the glucose level in the CAF group

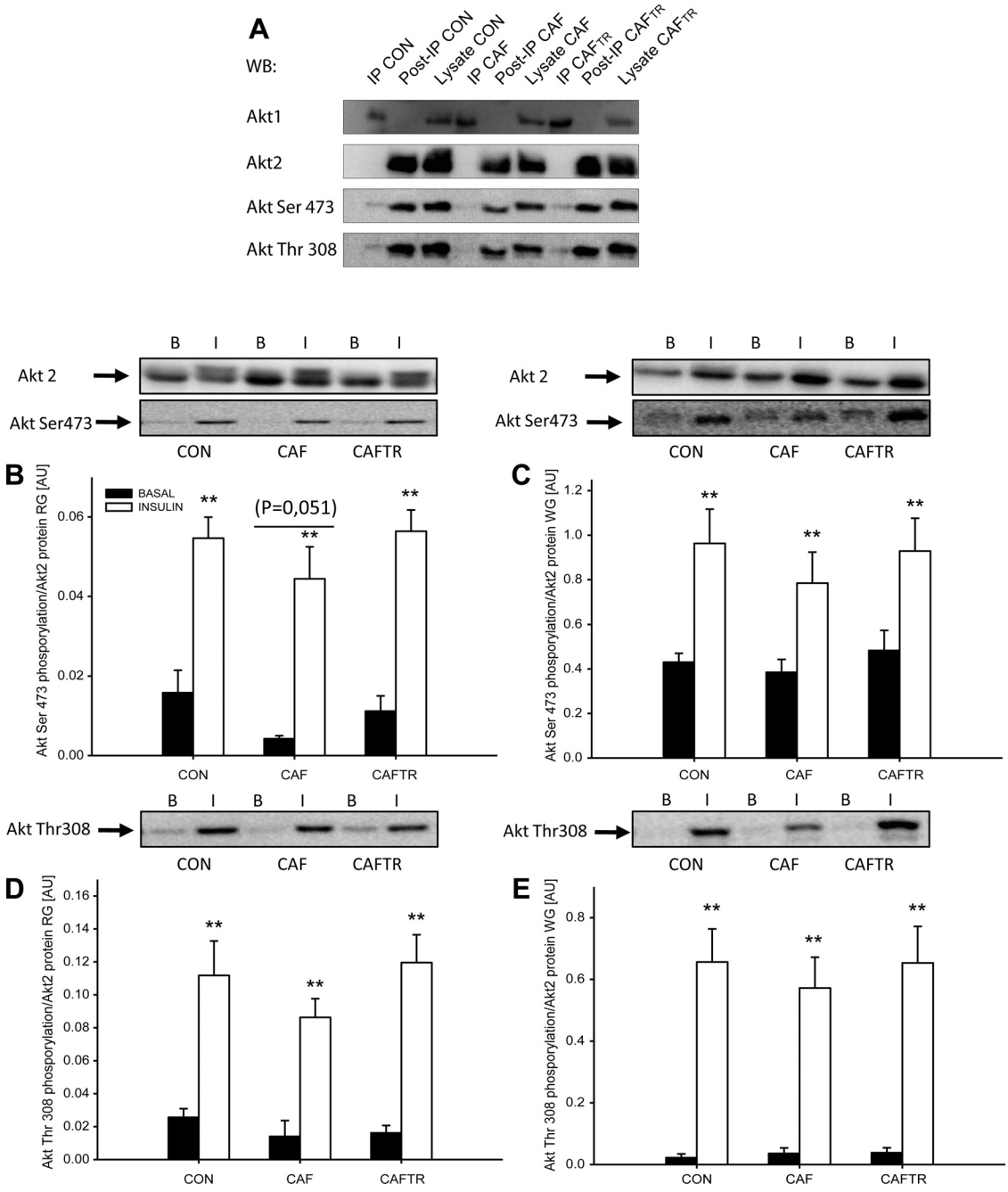

Fig. 4. *A*: Akt1 and Akt2 Ser^{473} and Thr^{308} phosphorylation in immunoprecipitates (IP) of Akt1 in lysates obtained from CON, CAF, and CAF_{TR} rats. IP, post-IP, and lysate from CON, CAF, and CAF_{TR} samples are loaded, and the protein content of Akt1 and Akt2 as well as phosphorylation on Ser^{473} and Thr^{308} is shown. Akt phosphorylation on Ser^{473} (*B* and *C*) and Thr^{308} (*D* and *E*) in the basal (black bars) and insulin-stimulated (open bars) state in RG (*B–D*) and WG (*C–E*) muscle from a perfused hindlimb. Muscles were obtained from CON rats and rats receiving cafeteria-style diet in the absence and in the presence of training interventions during the last 4 wk of the 12-wk diet period. Values are means ± SE; $n = 5–9$. $^{**}P < 0.01$, different compared with basal.

throughout the IVGTT was elevated (37) as well as when expressed as area under the curve (AUC; $P < 0.05$). Plasma insulin at all time points as well as when it was expressed as AUC was significantly higher ($P < 0.01$) in the CAF group compared with the two other groups (Fig. 1).

In Vitro Glucose Transport

Glucose transport in perfused muscle was similar between groups in the absence of insulin in both red (RG) and white (WG) gastrocnemius muscle (Fig. 2). In response to insulin, glucose uptake increased about fourfold ($P < 0.05$) in RG in the CON group, whereas no significant response was observed in the CAF group. Interestingly, the insulin response was rescued in the CAF_{TR} group in RG ($P < 0.05$). In WG the insulin response was less than in RG, and like in RG there was no response in the CAF group. In contrast to the results in RG, glucose transport only tended to be rescued by exercise in the CAF_{TR} group (Fig. 2).

Insulin Receptor Phosphorylation and PI 3-Kinase Activity

Basal phosphorylation of the insulin receptor (IR) in RG was equal among groups. Upon insulin stimulation, IR was activated via phosphorylation on Tyr^{1151}, which is one of the major autophosphorylation sites. Phosphorylation of IR increased ~1.5-fold ($P < 0.01$; Fig. 3) and was unaffected by the interventions.

IRS-1-associated PI 3-kinase activity in RG increased approximately onefold ($P < 0.01$; Fig. 3) upon insulin stimulation without any differences among groups in either the basal or the insulin-stimulated state.

JNK Phosphorylation

Phosphorylation of JNK Thr^{183}/Tyr^{185} in RG was not different among groups after 12 wk of interventions (Fig. 3).

Akt Isoforms

All Akt1 protein was immunoprecipitated with the Akt1 antibody, whereas all Akt2 was left in the postimmunoprecipitate. Both Ser^{473} and Thr^{308} phosphorylation were found solely in the postimmunoprecipitate, suggesting that only Akt2 was phosphorylated in the lysates (Fig. 4*A*). Therefore, phosphorylation of the two sites on Akt is expressed as phosphorylated protein relative to total Akt2 content.

Akt Phosphorylation

Basal phosphorylation of Akt on Ser^{473} and Thr^{308} was equal in all groups in both RG (Fig. 4, *B* and *D*) and WG (Fig. 4, *C* and *E*). Upon insulin stimulation, Akt was activated in terms of phosphorylation on both Ser^{473} and Thr^{308} in all groups. In RG, there was a tendency toward a minor reduction of Ser^{473} phosphorylation ($P = 0.051$) in the CAF group that was fully rescued by exercise in the CAF_{TR} group. Phosphorylation of Thr^{308} in RG was not affected; this was also the case for both phosphorylation sites in WG.

GSK-3 and TBC1D4 Phosphorylation

Basal GSK-3β Ser^{9} in RG did not differ among groups. In response to insulin stimulation, phosphorylation leading to inhibition increased by ~65% ($P < 0.01$; Fig. 5*A*) in all groups. In RG, TBC1D4 Thr^{642} phosphorylation was similar in all groups in the basal state. Upon insulin stimulation, phosphorylation level increased by ~70% ($P < 0.01$; Fig. 5*B*), with no statistical difference among groups.

GLUT4, Munc18c, and Syntaxin 4 Protein Content

Neither content of GLUT4, Munc18c, nor syntaxin 4 protein in RG differed among groups after 12 wk of interventions (Fig. 6). Since basal and insulin-stimulated samples did not differ, these are presented combined.

HKII and AMPK Protein Content and Phosphorylation

After 4 wk of exercise training the protein levels of HKII and AMPKα2 were increased in RG in the CAF_{TR} group by ~36 ($P < 0.05$; Fig. 7*A*) and 43% ($P < 0.01$; Fig. 7*C*), respectively, compared with both CON and CAF. There was no difference in AMPKα1 protein levels among groups (Fig. 7*B*). There was no effect of CAF diet on AMPK expression. Despite the increase in AMPKα2 expression in the CAF_{TR} group, the phosphorylation level of AMPK on Thr^{172} was not different among groups ($P = 0.172$; Fig. 7*D*). Since basal and insulin-stimulated samples did not differ, these are presented com-

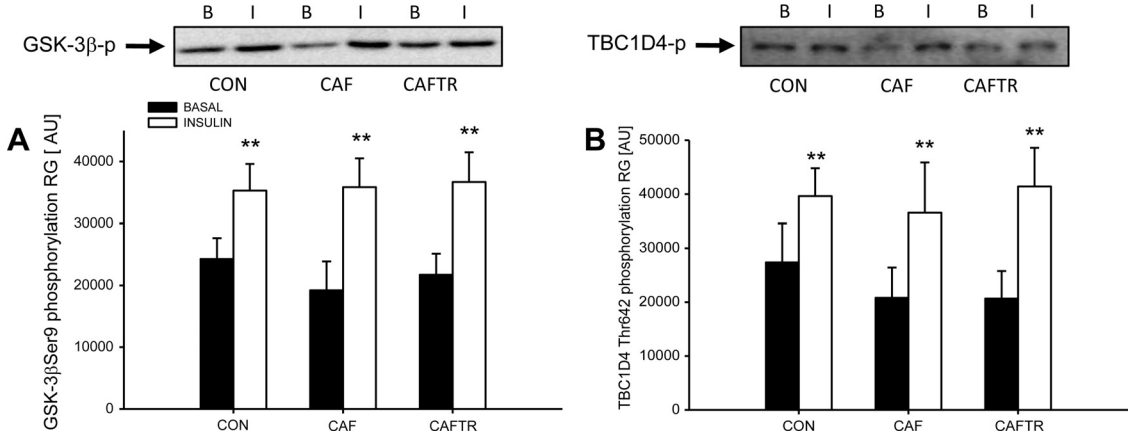

Fig. 5. Phosphorylation of GSK-3β Ser^{9} (*A*) and TBC1D4 Thr^{642} (*B*) in the B (black bars) and I (open bars) state in RG muscle obtained from CON, CAF, and CAF_{TR} rats. Values are means ± SE; $n = 6$–9. $^{**}P < 0.01$, different compared with basal.

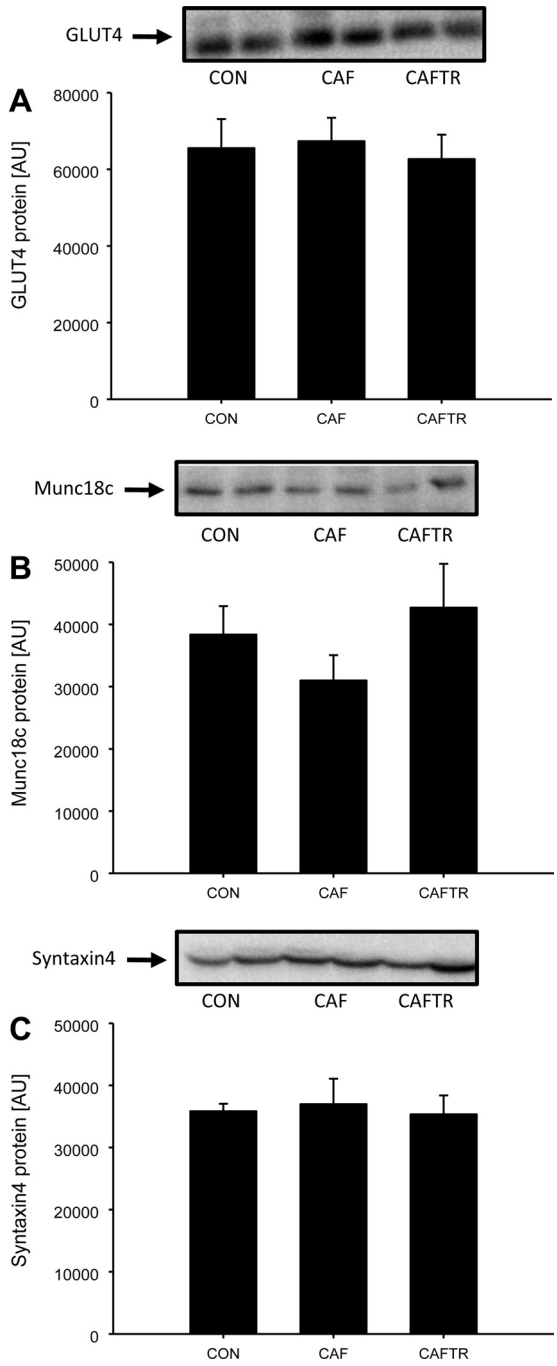

Fig. 6. Protein content of GLUT4 (*A*), Munc18c (*B*), and syntaxin 4 (*C*) in RG muscle obtained from CON, CAF, and CAF_{TR} rats. Values are means ± SE of combined basal and insulin-stimulated samples; $n = 11–13$.

bined. In WG there was no difference among groups for any of the mentioned parameters (data not shown).

DISCUSSION

The present study shows that CAF feeding leads to development of obesity and insulin resistance on the whole body and muscle level and that exercise training almost completely reverses these deleterious effects of the cafeteria diet. Surprisingly, CAF-induced insulin resistance was not accompanied by marked impairment of insulin signaling, activation of JNK, or altered expression or phosphorylation of AMPK in skeletal muscle.

Previously, CAF feeding in rats was shown to increase body weight considerably due to an increased daily energy intake and impair whole body glucose tolerance (25, 26). The impact of CAF feeding on insulin signaling toward an increased glucose uptake in skeletal muscle has not previously been addressed.

In contrast to previous studies where HFD is accompanied by activation of various stress-activated kinases and massive increases in IRS-1 serine phosphorylation, ~50% reduced IRS-1 tyrosine phosphorylation, and ~30% reduction in Akt serine phosphorylation/activity (36, 42), we observed no attenuations in the proximal part of the insulin-signaling cascade in terms of IR phosphorylation and IRS-1-associated PI 3-kinase activity in this study, whereas a minor defect was present on Akt phosphorylation. Since two downstream targets of Akt in the insulin-signaling cascade, GSK-3 and TBC1D4, appear to be regulated normally in all animals, the tendency toward reduced phosphorylation of Akt in the CAF group does not seem to play that important a role concerning regulation of insulin-stimulated glucose transport, in agreement with other studies showing that only very limited Akt phosphorylation is necessary to elicit a full response of glucose transport (10). Thus impaired insulin action caused by 12 wk of CAF seems to be due to defects distal to TBC1D4. This indicates that high-fat feeding apparently induces insulin resistance via different molecular mechanisms compared with insulin resistance induced by CAF feeding. CAF-induced obesity and insulin resistance seems to be a more relevant model that better resembles obesity and insulin resistance in a Western population since a normal Western diet does not contain 60% fat, as is often used in high-fat feeding (14a).

Previously, GLUT4 protein expression, as well as insulin-stimulated GLUT4 translocation to the plasma membrane, has been reported to be reduced in HFD (12, 18, 42). However, we found no effect of CAF feeding on GLUT4 protein expression. The diet-induced defect causing the reduced insulin-stimulated glucose transport might then be related to recruitment of GLUT4 to the plasma membrane and/or the docking/fusion process with the membrane. Soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins are central in fusion of transport vesicles with their target membrane. Munc18 proteins are thought to be involved in regulating conformation of syntaxins and, therefore, SNARE complex formation. In adipocytes, Munc18c has been shown to inhibit docking/fusion of GLUT4 vesicles by blocking binding of the v-SNARE VAMP2 to the t-SNARE syntaxin 4 (1, 3a, 34, 35). Furthermore, knockout of Munc18c in mice has been shown to increase insulin-stimulated GLUT4 exocytosis (13), whereas overexpression of Munc18c in adipocytes has been shown to inhibit insulin-stimulated GLUT4 translocation (35). In the present study, we found no effect of either CAF feeding or exercise training on Munc18c or syntaxin 4 protein expression, suggesting that these two proteins are not involved in the decreased insulin action after CAF.

Exercise training has previously been shown to protect against the deleterious effects of HFD on insulin-stimulated glucose disposal (15, 17, 18, 33). A period of exercise training in high-fat-fed rodents has been shown to reduce the diet-induced serine phosphorylation of IRS-1 and reverse the re-

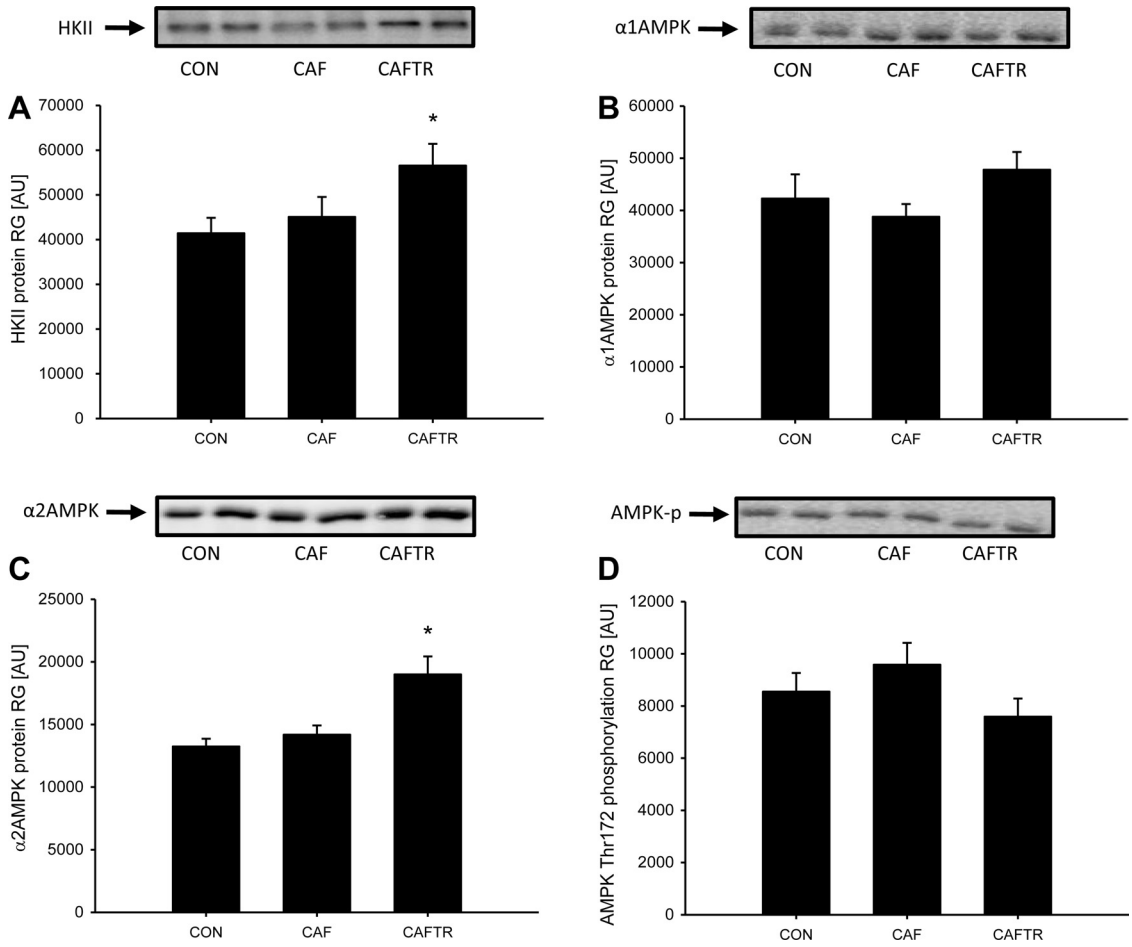

Fig. 7. Protein content of hexokinase II (HKII; *A*), AMP-activated protein kinase (AMPK)α1 (*B*), and AMPKα2 (*C*) as well as AMPK Thr^{172} phosphorylation (*D*) in RG muscle obtained from CON, CAF, and CAF$_{TR}$. Values are means ± SE of combined basal and insulin-stimulated samples; n = 11–17. *$P < 0.05$, different compared with CON.

duction in IRS-1-associated PI 3-kinase activity and the reduction in both phosphorylation and activity of cytosolic Akt2 (18, 42). However, the molecular mechanism behind the effect of exercise is unresolved. One possibility is that activation of AMPK during exercise (40) plays a major role as suggested (28). Supporting such a contention, obese Zucker rats as well as *ob/ob* mice treated with the AMPK-activating agent AICAR improved their metabolic status markedly (27, 30). Furthermore, incubation with palmitate has been shown to impair in vitro glucose transport in incubated rat extensor digitorum longus muscle, where AICAR treatment ameliorated palmitate-induced insulin resistance (24). Further supporting a possible role of AMPK in exercise-induced reversal of insulin resistance are findings in humans that showed that endurance training led to an increase in AMPKα1 expression accompanied by increases in both α1- and α2-associated AMPK activity and phosphorylation (6). Previously, exercise training following HFD has been shown to increase both α1-associated AMPK activity and protein expression, whereas α2-associated AMPK activity and protein expression was increased by HFD with no further effect of training (18). In the present study, AMPKα2 expression increased with training, whereas activity of AMPK in resting muscle as evaluated by AMPK phosphorylation was not increased. Thus it is unclear whether AMPK played a role in the reversal of insulin resistance with exercise.

Previous studies have shown conflicting data concerning activation, phosphorylation, and expression of AMPK following HFD. Some show an increased activation and phosphorylation of AMPKα2 but not of AMPKα1 (18), whereas others show a decrease in AMPKα2 but not of AMPKα1 protein expression accompanied by a decreased phosphorylation of AMPK (19). In the present study we found no effect of CAF on either AMPK expression or phosphorylation.

In conclusion, we have provided novel data to demonstrate that excess intake of a palatable low-fat diet induces obesity and insulin resistance both on a whole body level and in skeletal muscle. Interestingly, CAF-induced insulin resistance was not accompanied by marked impairment of insulin signaling and does not seem to be related to low-grade inflammation, as has been described after HFD. Thus the present results indicate that the mechanism by which insulin resistance is induced by a CAF is different from insulin resistance induced by a high-fat diet and is furthermore reversed by exercise training.

ACKNOWLEDGMENTS

We thank Jesper Birk and Monique Ramaekers for skilled technical assistance. We also thank D. David James, Garvan Institute of Medical Research, for the kind donation of TBC1D4 Thr^{642} antibody and Grahame Hardie, University of Dundee, for the kind donation of AMPKα1 and -α2 antibodies.

GRANTS

This study was supported by grants from the Danish Medical Research Council, the Novo Nordisk Foundation, the Lundbeck Foundation, the Copenhagen Muscle Centre Research Centre, the "Onderzoeksraad Katholieke Universiteit-Leuven" (grant no. OT04/45), and the "Fonds voor Wetenschappelijk Onderzoek Vlaanderen" (grant no. G.0233.05).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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